

# CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

## EDITORIAL BOARD

Frederick M. Ausubel  
Massachusetts General Hospital & Harvard Medical School

Roger Brent  
Massachusetts General Hospital & Harvard Medical School

Robert E. Kingston  
Massachusetts General Hospital & Harvard Medical School

David D. Moore  
Massachusetts General Hospital & Harvard Medical School

J.G. Seidman  
Harvard Medical School

John A. Smith  
University of Alabama

Kevin Struhl  
Harvard Medical School

## GUEST EDITORS

Lisa M. Albright  
DNA Sequencing

Donald M. Coen  
Harvard Medical School  
Polymerase Chain Reaction

Ajit Varki  
University of California San Diego  
Glycoproteins

## SERIES EDITOR

Virginia Benson Chanda



BEST AVAILABLE COPY

John Wiley & Sons, Inc.

CORE 13 (535)

Copyright © 1994–1997 by John Wiley & Sons, Inc.

Copyright © 1987–1994 by Current Protocols

All rights reserved. Published simultaneously in Canada.

Reproduction or translation of any part of this work beyond that permitted by Section 107 or 108 of the 1976 United States Copyright Act without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to the Permissions Department, John Wiley & Sons, Inc.

While the authors, editors, and publisher believe that the specification and usage of reagents, equipment, and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. This is particularly important in regard to new or infrequently employed chemicals or experimental reagents.

*Library of Congress Cataloging in Publication Data:*

Current protocols in molecular biology. 3 vols.

1. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.C87 1987 574.53'028 87-21033  
ISBN 0-471-50338-X

Printed in the United States of America

20 19 18 17 16 15 14 13

BEST AVAILABLE COPY

**SDS electrophoresis buffer, 5×**

15.1 g Tris base

72.0 g glycine

5.0 g SDS

H<sub>2</sub>O to 1000 ml

Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4° C until use (up to 1 month).

**SED (standard enzyme diluent)**

20 mM Tris-Cl, pH 7.5

500 µg/ml bovine serum albumin (Pentax Fraction V)

10 mM 2-mercaptoethanol

Store up to 1 month at 4° C

**Sodium acetate, 3 M**

Dissolve 408 g sodium acetate-3H<sub>2</sub>O in 800 ml H<sub>2</sub>O

Add H<sub>2</sub>O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

**Sodium acetate buffer, 0.1 M**

**Solution A:** 11.55 ml glacial acetic acid/liter (0.2 M).

**Solution B:** 27.2 g sodium acetate (NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 100 ml. (See Potassium acetate buffer recipe for further details.)

**Sodium phosphate buffer, 0.1 M**

**Solution A:** 27.6 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter (0.2 M).

**Solution B:** 53.65 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

**SSC (sodium chloride/sodium citrate), 20×**

3 M NaCl (175 g/liter)

0.3 M Na<sub>3</sub>citrate-2H<sub>2</sub>O (88 g/liter)

Adjust pH to 7.0 with 1 M HCl

**STE buffer**

10 mM Tris-Cl, pH 7.5

10 mM NaCl

1 mM EDTA, pH 8.0

**TAE (Tris/acetate/EDTA) electrophoresis buffer**

**50× stock solution:**

242 g Tris base

57.1 ml glacial acetic acid

37.2 g Na<sub>2</sub>EDTA-2H<sub>2</sub>O

H<sub>2</sub>O to 1 liter

**Working solution, pH ~8.5:**

40 mM Tris-acetate

2 mM Na<sub>2</sub>EDTA-2H<sub>2</sub>O

**TBE (Tris/borate/EDTA) electrophoresis buffer**

**10× stock solution, 1 liter:**

108 g Tris base (890 mM)

55 g boric acid (890 mM)

40 ml 0.5 M EDTA, pH 8.0 (20 mM)